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EXTRACELLULAR MATRIX PROTEOGLYCANS AND CELL–SUBSTRATUM ADHESION OF HUMAN ENDOTHELIAL CELLS: THE EFFECT OF METHYL β-D-XYLOPYRANOSIDE*

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ABSTRACT

The influence of methyl β-D-xylopyranoside on human endothelial cell proteoglycans isolated from the medium and extracellular matrix was investigated. Confluent cultures of human endothelial cells incorporate significant amounts of heparan sulfate (78%), chondroitin sulfate (10%), and dermatan sulfate (12%) into the extracellular matrix. Chondroitin sulfate (35%) and dermatan sulfate (37%) were the major glycosaminoglycans present in the medium. In the presence of methyl β-D-xylopyranoside, incorporation of labeled proteoglycans into extracellular matrix was diminished by ~70%. Heparan sulfate comprised the major proteoglycan present in extracellular matrix (89%) in cells grown in the presence of methyl β-D-xylopyranoside. In contrast to the incorporation of proteoglycan into extracellular matrix, methyl β-D-xylopyranoside stimulated the secretion of labeled glycosaminoglycan chains into the medium 2.5-fold. In the presence of methyl β-D-xylopyranoside, secretion of chondroitin sulfate into the medium was markedly stimulated, with a slight increase in secretion of heparan sulfate. Chondroitin sulfate (62%) and heparan sulfate (34%) were the major labeled glycosaminoglycans present in medium from methyl β-D-xylopyranoside-treated cultures. The effect of methyl β-D-xylopyranoside on cell adhesion and detachment was investigated. Cell detachment from extracellular matrix depleted of proteoglycan was significantly faster than detachment from normal matrix. Conversely, human endothelial cells adhered faster to normal matrix than to matrix depleted of proteoglycan.

INTRODUCTION

Cultured endothelial cells retain the ability to synthesize and secrete basement-membrane components that organize into a fibrillar network similar to that

*Dedicated to Roger W. Jeanloz.

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observed *in vivo*[1–3]. Proteoglycans, which have been implicated in cell adhesion, are important structural components of the extracellular matrix[4–7].

Several studies have demonstrated that the composition of the extracellular matrix may influence both qualitatively and quantitatively the synthesis of new extracellular matrix, as well as cell–substratum adhesion and detachment[8–11]. Human pulmonary endothelial cells synthesize different proportions of glycosaminoglycans when grown on collagen or smooth muscle cell-derived extracellular matrix, as compared to plastic[8]. Robinson and Gospodarowicz[9] and Tseng *et al.*[10] have demonstrated that the glycosaminoglycans and collagen synthesized by bovine corneal endothelial cells was dependent upon the growth state of the cells, the presence of fibroblast growth-factor, and the type of substrate on which the cells were grown. Haas *et al.*[11] have demonstrated that the composition of the extracellular matrix modulates cell morphology and the composition of cellular-adhesion sites. We have demonstrated that human endothelial cells grown on matrix containing small amounts of labeled glycosaminoglycans incorporate smaller amounts of glycosaminoglycans into extracellular matrix and detach more rapidly from the substratum compared to cells grown on matrix containing larger amounts of labeled glycosaminoglycans[12].

Several studies have demonstrated that, in the presence of β-D-xylopyranosides, the relative amounts of proteoglycan present in extracellular matrix or secreted into the medium can be altered. Cells exposed to β-D-xylopyranosides synthesize glycosaminoglycan chains onto the exogenous β-D-xylopyranoside instead of the normal endogenous acceptor, the xylopyranosyl-substituted core protein[13–15]. In this study, we have examined the effect of methyl β-D-xylopyranoside on the incorporation of human endothelial cell proteoglycans into extracellular matrix. We have demonstrated that the composition of labeled proteoglycan in the extracellular matrix was altered in the presence of methyl β-D-xylopyranoside. The altered extracellular matrix modulated endothelial-cell adhesion and detachment.

EXPERIMENTAL

Materials. — D-[1,6-^{3}H]Glucosamine (30–60 Ci/mmol), $Na_2{}^{35}SO_4$ (10–1000 Ci/mmol), L-[2,3-^{3}H]proline (15–30 Ci/mmol) and Riafluor were from New England Nuclear. Collagenase type I *(Clostridium histolyticum)* and crystalline trypsin (210 U/mg) were supplied by Worthington Millipore Corp., Freehold, NJ. Pronase *(Streptomyces griseus)* and *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) were from Calbiochem–Behring Corp., La Jolla, CA. Bovine serum albumin (BSA), gelatin (swine skin) type IV, and methyl β-D-xylopyranoside were obtained from Sigma Chemical Co., St. Louis, MO. Guanidine hydrochloride was supplied by Research Plus Laboratories, Inc. Tissue culture supplies: Medium 199, newborn bovine serum, and L-glutamine were supplied by Grand Island Biological Co., Grand Island, NY; tissue-culture dishes and conical centrifuge-tubes were

from Falcon Products. Chondroitin mucoprotein was purchased from ICN Pharmaceuticals, Inc.; hyaluronic acid, disaccharide standards and chondroitinase ABC and AC were purchased from Miles Laboratories. Precoated thin-layer cellulose acetate sheets without fluorescent indicator were purchased from E. Merck Reagents, Darmstadt, West Germany. Sepharose CL-4B and -6B were from Pharmacia Fine Chemicals, Uppsala, Sweden. Molecular weight standards: myosin heavy chain, 205,000 daltons; phosphorylase b, 97,000 daltons; ovalbumin, 45,000 daltons; chymotrypsinogen A, 25,000 daltons; and cytochrome c, 12,500 daltons were the generous gift of Dr. Lawrence Rosenberg, Albert Einstein College of Medicine.

Isolation of human endothelial cell-growth factor. — Human endothelial-cell growth factor was isolated as described previously by Gordon *et al.*[16].

Culture conditions. — Endothelial cells of human umbilical vein were isolated according to the method of Jaffe[17] and grown as described by Gordon *et al.*[16]. Ascorbate (50 μg/mL) was added to cell cultures fresh daily. Cells were plated grown to confluence in medium containing various concentrations of methyl β-D-xylopyranoside (0.1–5.0mM). In these experiments, cells were grown continuously in the presence of methyl β-D-xylopyranoside and passaged twice in the presence of the same concentration of methyl β-D-xylopyranoside as used at the time of plating. Control cells were grown without methyl β-D-xylopyranoside.

Metabolic labeling of endothelial cell cultures. — Endothelial cells were grown to confluence as described, and labeling was initiated by the addition of fresh medium containing either 5 μCi/mL [^{3}H]glucosamine and 10 μCi/mL $Na_2{}^{35}SO_4$, or 10 μCi/mL $Na_2{}^{35}SO_4$ and 5 μCi/mL [^{3}H]proline. Cultures grown with methyl β-D-xylopyranoside contained the same concentration of methyl β-D-xylopyranoside during the labeling period. Cells were labelled for 96 h as described previously[18].

Isolation of labeled proteoglycans. — Labeled proteoglycans were obtained from medium and extracellular matrix in the following manner. At the end of the radiolabeling period, medium was removed and dry guanidine hydrochloride added for a final concentration of 4M and the solution adjusted to 0.1M Na_2EDTA, 0.05M sodium acetate, 2mM phenylmethylsulfonyl fluoride, pH 6.3, (4M guanidine · HCl extracting buffer) for 24 h at 4° (ref. 19). Cell monolayers were washed twice in the Hepes buffer, pH 7.55, and extracellular matrix isolated with 0.5% Triton X-100 and 0.02M NH_4OH to solubilize cells as described by Greenburg and Gospodarowicz[20]. Extracellular matrix was washed three times with H_2O to remove cell debris, and incubated in 4M guanidine·HCl extracting buffer containing 0.5% Triton X-100 and stored for 24 h at 4°. All fraction extracts were clear, and centrifugation at 15,000 r.p.m. for 30 min at 4° did not produce a pellet. An aliquot of the medium and extracellular matrix fractions were dialyzed against 4M guanidine·HCl containing protease inhibitors for 48 h, at 4°. In some experiments, an aliquot of the medium and extracellular matrix fractions were lyophilized and stored at −20°.

Proteoglycans were isolated under dissociative conditions. An aliquot of the extracellular matrix or medium fraction was counted for radioactivity and 2 mL

loaded onto a column (1.5 × 240 cm) of Sepharose CL-4B equilibrated in and eluted with 4M guanidine·HCl containing 0.15M sodium acetate, pH 6.3, at 23°. Fractions (2.7 mL) were collected and an aliquot of each fraction counted for radioactivity. Recovery of radioactivity from the column was ~95%. Molecular weights of individual proteoglycan peaks were determined from a standard curve of log(molecular weights) *vs.* elution volume generated by standards run under identical conditions.

Isolation and characterization of glycosaminoglycans. — Labeled proteoglycans isolated after column chromatography on Sepharose CL-4B were treated with 0.02M sodium hydroxide and M sodium borohydride, for 24 h at 45°, to cleave intact oligosaccharides from the core protein by β-elimination[21]. The samples were made neutral with acetic acid and the molecular weights of alkaline borohydride digests were determined on a column of Sepharose CL-4B equilibrated in and eluted with 4M guanidine·HCl containing 0.15M sodium acetate, pH 6.3, at 23°.

The composition of the glycosaminoglycans from medium and extracellular matrix was determined after pronase digestion[12,18] or alkaline borohydride treatment, by digestion with chondroitinase ABC or AC as described by Gordon *et al.*[12,18].

Heparan sulfate was determined by nitrous acid deamination according to the method of Rollins and Culp[22]. Confluent cultures were metabolically labeled with 5 μCi/mL [^{3}H]glucosamine and 2 μCi/mL $Na_2{}^{35}SO_4$ and labeled cells and substrate-attached material were isolated and digested with pronase and chondroitinase ABC as described. Lyophilized chondroitinase digests were resuspended in 2 mL of 150mM Tris–HCl, pH 7.4, and divided into two equal (1-mL) aliquots. The first aliquot was applied to a column (1.5 × 100 cm) of Sepharose CL-6B and eluted at 23° with 150mM Tris–HCl (pH 7.4) containing 0.2% sodium dodecyl sulfate. The column was eluted at a rate of 5 mL/h, 1.6-mL fractions were collected and counted in a liquid-scintillation counter, and a profile of radioactivity *vs.* fraction number obtained. The second aliquot was treated with 300 μL of 18% $NaNO_2$ in 1.8M acetic acid for 1 h at 23°. The reaction was terminated by the addition of 2M NaOH until the samples were neutral. Reaction products were separated by column chromatography as described, fractions counted in a liquid-scintillation counter, and the profile obtained compared to that obtained from the untreated sample. Heparan sulfate was detected as radioactivity in the chondroitinase digest susceptible to nitrous acid, and was determined as the difference in c.p.m. between the radioactive profile obtained before and after treatment with nitrous acid.

Endothelial-cell detachment assay. — Endothelial cells were grown to confluence in the absence or presence of 3.0mM methyl β-D-xylopyranoside and a detachment assay performed using trypsin as described by Gordon *et al.*[12].

Endothelial-cell adhesion assay. — Extracellular matrix from cells grown to confluence in the presence or absence of 3.0mM methyl β-D-xylopyranoside was isolated by 0.5% Triton X-100 and 0.02M NH_4OH, and an adhesion assay performed as described by Gordon *et al.*[12].

RESULTS

Effect of methyl β-D-xylopyranoside on synthesis of labelled glycosaminoglycan. — The effect of methyl β-D-xylopyranoside on incorporation of labeled proteoglycan into extracellular matrix or secretion into the medium was investigated. Cells were plated and grown for 2 passages in 0.1–5.0mM methyl β-D-xylopyranoside. Control cultures were grown in the absence of methyl β-D-xylopyranoside. Ascorbate was added to all cultures to allow for proper deposition of collagen[23]. Labeled glycosaminoglycan were isolated by alkaline borohydride digestion from metabolically labeled cultures. Addition of methyl β-D-xylopyranoside to cultures did not affect the rate of cell proliferation or morphology. Cells grown in the presence or absence of methyl β-D-xylopyranoside reached confluence at similar rates.

Cultures grown in the presence of 0.25mM methyl β-D-xylopyranoside exhibited a 10–30% reduction in the amount of labeled glycosaminoglycans incorporated into extracellular matrix as compared to that incorporated in control cultures. Concentrations of methyl β-D-xylopyranoside less than 0.1mM had no appreciable effect on incorporation of labeled proteoglycan into extracellular matrix (data not shown). Addition of 0.5mM methyl β-D-xylopyranoside to cultures resulted in a 70% decrease in the amount of labeled glycosaminoglycan incorporated into extracellular matrix as compared to that incorporated in control cultures (Fig. 1). This effect was maximal at 0.5mM, and higher concentrations of methyl β-D-xylopyranoside did not further inhibit incorporation of labeled glycosaminoglycan into extracellular matrix.

In contrast to glycosaminoglycan incorporation into extracellular matrix, increasing concentrations of methyl β-D-xylopyranoside of 0.5–2.0mM stimulated secretion of labeled glycosaminoglycans into the medium 2.5-fold as compared to cultures grown without methyl β-D-xylopyranoside (Fig. 1). All subsequent experiments were performed with 3.0mM methyl β-D-xylopyranoside.

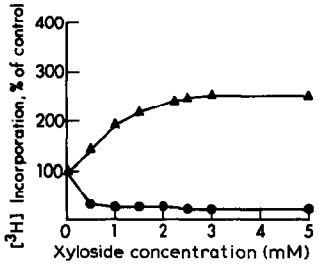

Fig. 1. Effect of methyl β-D-xylopyranoside on incorporation of [3H]glycosaminoglycan into extracellular matrix and secretion into the medium. Cultures grown in the presence or absence of various concentrations were metabolically labeled and labeled glycosaminoglycan isolated from (●) extracellular matrix or (▲) medium as described. Results expressed as the percentage of total glycosaminoglycan isolated from cultures grown without methyl β-D-xylopyranoside.

Isolation and characterization of labeled proteoglycan synthesized in the absence or presence of methyl β-D-xylopyranoside. — Labeled proteoglycans were isolated from cultures grown in the presence or absence of 3.0mM methyl β-D-xylopyranoside and metabolically labeled with [^{3}H]glucosamine and $Na_2{}^{35}SO_4$. The molecular weights of labeled proteoglycans isolated by column chromatography on Sepharose CL-4B from medium and extracellular-matrix fractions from control cultures were compared with those from comparable fractions from methyl β-D-xylopyranoside-treated cultures. Medium from control cultures contained two labeled proteoglycan species having K_{av} 0.23 and 0.77, corresponding to approximate molecular weights of 720,000 and 10,000 daltons, respectively (Fig. 2B). Medium from methyl β-D-xylopyranoside-treated cultures contained two small peaks of radioactivity K_{av} 0.24 and 0.60, corresponding to approximate molecular weights of 690,000 and 40,000. Most of the labeled material, however, was contained in a third peak eluting in the included volume of the column (Fig. 2A). An aliquot of this third peak was chromatographed on a column of Sepharose CL-6B before and after pronase digestion. The elution position of this labeled material was not altered by pronase digestion and the material was assumed to be glycos-

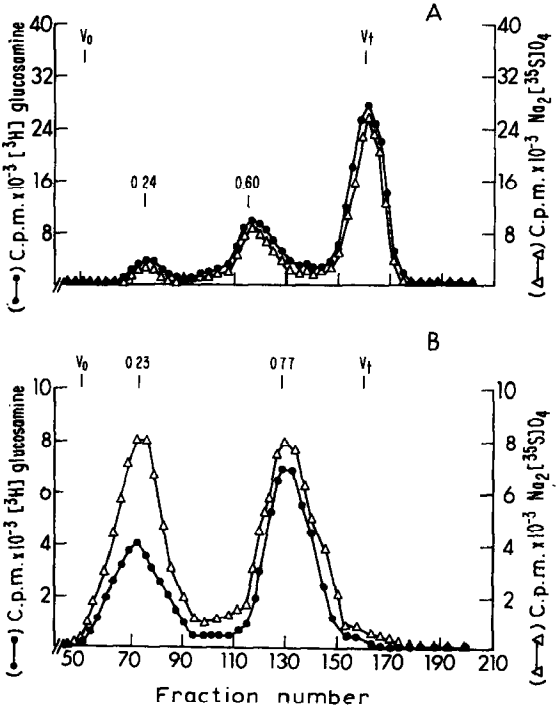

Fig. 2. Comparison of labeled proteoglycans isolated from medium of control and methyl β-D-xylopyranoside-treated cultures. Endothelial cell cultures were metabolically labeled and labeled proteoglycans isolated from medium from methyl β-D-xylopyranoside-treated (A) and control (B) cultures by column chromatography on Sepharose CL-4B. Fractions (2.7 mL) were collected and an aliquot sampled for [^{3}H] (●) and [^{35}S] (△).

aminoglycan chains (data not shown). Thus, medium from methyl β-D-xylopyranoside-treated cultures contained a significant amount of free glycosaminoglycan chains (or chains attached to very small amounts of protein) not present in medium from control cultures. In addition, the largest molecular-weight proteoglycan species isolated from medium from methyl β-D-xylopyranoside-treated cultures was of slightly smaller molecular weight than that isolated from medium from control cultures.

Extracellular matrix from control cultures contained labeled proteoglycan species having K_{av} 0.47, corresponding to an approximate molecular weight of 110,000 daltons (Fig. 3A). Extracellular matrix from methyl β-D-xylopyranoside-treated cultures contained proteoglycan species eluting with K_{av} 0.56, corresponding to an approximate molecular weight of 50,000 daltons (Fig. 3B). Thus, extracellular matrix from methyl β-D-xylopyranoside-treated cultures contained labeled proteoglycan species of smaller molecular weight than labeled proteoglycans from extracellular matrix of control endothelial cells.

In all experiments, ^{3}H- and ^{35}S-material co-migrated with comparable incorporation of both radiolabels. Incorporation of labeled proteoglycan into extracellular matrix of methyl β-D-xylopyranoside-treated cultures was, however, signifi-

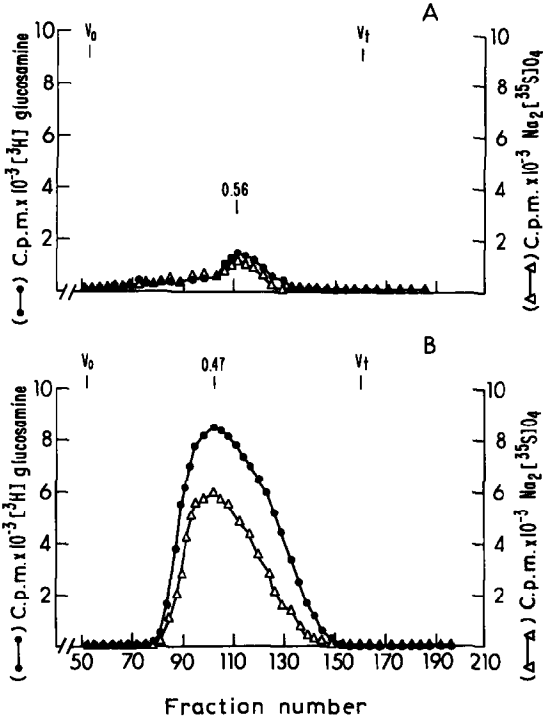

Fig. 3. Comparison of labeled proteoglycans isolated from extracellular matrix of control and methyl β-D-xylopyranoside-treated cultures. Figure legend as in Fig. 2; (A) methyl β-D-xylopyranoside-treated cultures, (B) control cultures.

cantly diminished (compare Fig. 3A and B). These experiments and those represented in Fig. 1 demonstrate that total incorporation of labeled proteoglycan into extracellular matrix in methyl β-D-xylopyranoside-treated cultures was ~30% of that incorporated into extracellular matrix in control cultures.

An aliquot of labeled proteoglycans from extracellular matrix of control and methyl β-D-xylopyranoside-treated cultures was digested with alkaline borohydride. The alkaline borohydride digest of labeled proteoglycans from extracellular matrix from control cultures could be resolved by column chromatography on Sepharose CL-4B into two peaks of radioactivity of K_{av} 0.78 and 0.95, corresponding to approximate molecular weights of 10,000 and 4,000 daltons, respectively (Fig. 4B). Alkaline borohydride digests of labeled proteoglycans from extracellular matrix from methyl β-D-xylopyranoside-treated cultures could be resolved into one peak of radioactivity with K_{av} 0.65, corresponding to an approximate molecular weight of 25,000 daltons (Fig. 4A).

The composition of glycosaminoglycan chains from medium and extracellular matrix of control and methyl β-D-xylopyranoside-treated cultures was determined by enzymic degradation with chondroitinase ABC or AC as described in Methods. Dermatan sulfate was determined as the difference in radioactivity between chondroitinase AC-resistant material and chondroitinase ABC-susceptible material. Heparan sulfate was determined as chondroitinase ABC-resistant material susceptible to nitrous acid deamination[12,16].

Medium from control cultures contained chondroitin sulfate and dermatan sulfate in about equal amounts (Table I). The amount of chondroitin sulfate present refers to the total amount of chondroitin 4-sulfate and chondroitin 6-sulfate. About

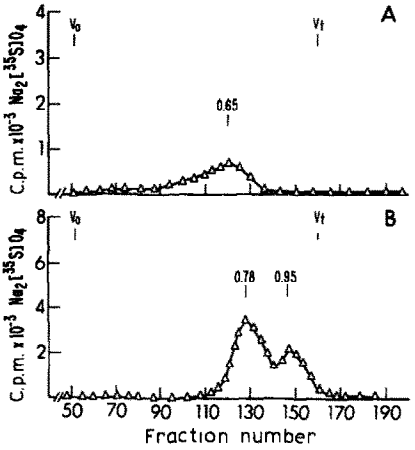

Fig. 4. Alkaline borohydride digestion of labeled proteoglycans from extracellular matrix of control and methyl β-D-xylopyranoside-treated cultures. The labeled proteoglycan fractions isolated after Sepharose CL-4B chromatography of methyl β-D-xylopyranoside-treated (A) and control (B) cultures were treated with alkaline borohydride as described in Methods and rechromatographed on a column of Sepharose-CL-4B. Fractions (2.7 mL) were collected and an aliquot sampled for [^{35}S] (△).

30% of the labeled glycosaminoglycan present in medium was heparan sulfate (Table I). Chondroitin sulfate comprised the major glycosaminoglycan (62%) in medium from methyl β-D-xylopyranoside-treated cultures, with heparan sulfate comprising ~30%. Medium from cultures exposed to methyl β-D-xylopyranoside contained little dermatan sulfate (Table I).

Heparan sulfate was the major glycosaminoglycan identified in extracellular matrix in both control and methyl β-D-xylopyranoside-treated cultures (78 and 89%, respectively). Cells grown in the absence of methyl β-D-xylopyranoside incorporated about equal amounts of chondroitin sulfate and dermatan sulfate into extracellular matrix (Table I). In contrast, methyl β-D-xylopyranoside inhibited incorporation of dermatan sulfate into extracellular matrix (Table I).

Effect of methyl β-D-xylopyranoside on the incorporation of [^{3}H]proline [^{35}S]sulfate into extracellular matrix. — Confluent cultures of human endothelial cells grown in the presence of ascorbate, with or without methyl β-D-xylopyranoside, were metabolically labeled with [^{3}H]proline and $Na_2{}^{35}SO_4$. Extracellular matrix was isolated and an aliquot analyzed for ^{3}H and ^{35}S. Control cells incorporated ~1.5-fold more [^{3}H]proline and ~4-fold more ^{35}S-material into extracellular matrix as methyl β-D-xylopyranoside-treated cultures, based on c.p.m./10^6 cells (Table II).

Effect of methyl β-D-xylopyranoside on cell adhesion and detachment. — We have demonstrated that human endothelial cells are relatively more resistant to detachment from matrix containing large amounts of labeled glycosaminoglycan than from matrix containing relatively smaller amounts of labeled glycosaminoglycan[12]. Studies were performed to compare cell–substratum detachment from, and cell adhesion to, normal extracellular matrix and proteoglycan-depleted matrix.

Endothelial cells were grown to confluence either in the presence or absence of 3.0mM methyl β-D-xylopyranoside and a detachment assay performed as described (Fig. 5). Cells grown in the presence of 3.0mM methyl β-D-xylopyranoside

TABLE I

GLYCOSAMINOGLYCAN COMPOSITION OF PROTEOGLYCAN ISOLATED FROM MEDIUM AND EXTRACELLULAR MATRIX OF CONTROL AND METHYL β-D-XYLOPYRANOSIDE-TREATED CULTURES[a]

	Medium		*Extracellular matrix*	
Glycosaminoglycan	*Control*	*Xylopyranoside*	*Control*	*Xylopyranoside*
Heparan sulfate[b]	28[c]	34	78	89
Chondroitin sulfate[d]	35	62	10	11
Dermatan sulfate[e]	37	4	12	N.D.[f]

[a]Control and methyl β-D-xylopyranoside-treated cultures were metabolically labeled and glycosaminoglycans isolated as described. [b]Chondroitinase ABC-resistant, nitrous acid sensitive. [c]Results expressed as percentage of the total counts per min in the glycosaminoglycan fraction isolated. [d]Chondroitinase ABC- and AC-sensitive. [e]Chondroitinase AC-resistant, chondroitinase ABC-sensitive. [f]N.D. = not detectable.

TABLE II

EFFECT OF METHYL β-D-XYLOPYRANOSIDE ON INCORPORATION OF [^{3}H]PROLINE AND $Na_2{}^{35}SO_4$ INTO EXTRACELLULAR MATRIX[a]

	[^{3}H]Proline c.p.m./10^6 Cells	*$Na_2{}^{35}SO_4$ c.p.m./10^6 Cells*
Control extracellular matrix	27,661	38,442
Xylopyranoside treated Extracellular matrix	18,684	8,884

[a]Control and methyl β-D-xylopyranoside-treated cultures were metabolically labeled with [^{3}H]proline and $Na_2{}^{35}SO_4$. Extracellular matrix was isolated as described and an aliquot sampled for ^{3}H and ^{35}S.

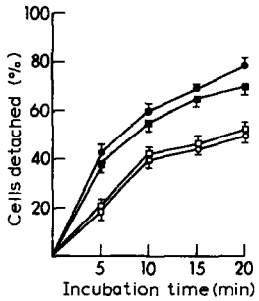

Fig. 5. Effect of methyl β-D-xylopyranoside on the detachment of human endothelial cells. Human endothelial cells were grown in the presence or absence of 3.0mM methyl β-D-xylopyranoside and ascorbate and a detachment assay performed as described. Detachment of (●) cells grown in the presence of 3.0mM methyl β-D-xylopyranoside or (■) cells grown in the presence of 3.0mM methyl β-D-xylopyranoside and 50 μg/mL ascorbate was compared with detachment of control cultures, (○) grown in the absence of methyl β-D-xylopyranoside or ascorbate, or (□) cells grown in the absence of methyl β-D-xylopyranoside and with ascorbate. Results expressed as the percentage of cells that detached over 20 min. Results are the mean ±standard error for three experiments.

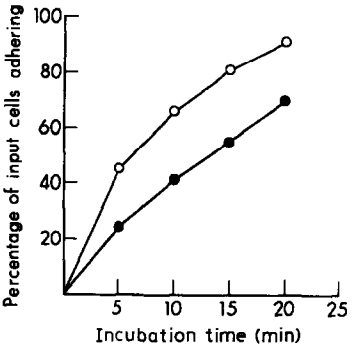

Fig. 6. Effect of methyl β-D-xylopyranoside on human endothelial-cell adhesion. Extracellular matrix was isolated from cells grown in the presence (●) or absence (○) of 3.0mM methyl β-D-xylopyranoside and an adhesion assay performed as described. Results expressed as the percentage of input cells adhering over 20 min. Results are the mean ±standard error for three experiments.

detached at a significantly higher initial rate from the substratum than cells grown in the absence of methyl β-D-xylopyranoside. The percentage of cells detached over 20 min was higher than for cells grown in the absence of methyl β-D-xylopyranoside (Fig. 5).

To test adhesion of cells to control or proteoglycan-depleted matrix, extracellular matrix was isolated from cultures grown in the absence or presence of 3.0mM methyl β-D-xylopyranoside, and an adhesion assay performed as described in Methods. The rate of cell adhesion to a proteoglycan-depleted matrix was lower than the rate of cell adhesion to normal matrix. The percentage of cells adhering to normal matrix was higher than the percentage of cells adhering to proteoglycan-depleted matrix over the same time interval (Fig. 6).

DISCUSSION

Cultured vascular endothelial cells actively synthesize and secrete extracellular-matrix components, which organize into basement membrane similar to that observed *in vivo*. We have previously demonstrated that, in the presence of an endothelial cell-growth factor, human endothelial cells incorporate significant amounts of labeled chondroitin sulfate and dermatan sulfate into extracellular matrix[18]. β-D-Xylopyranosides initiate synthesis of glycosaminoglycan onto the exogenous methyl β-D-xylopyranoside instead of the endogenous acceptor, the core protein[13–15]. The effect of methyl β-D-xylopyranoside on incorporation of this xylopyranosyl-linked class of glycosaminoglycans into extracellular matrix has been investigated.

Methyl β-D-xylopyranoside-treated cultures incorporated only ~30% of the amount of labeled proteoglycan into extracellular matrix as that incorporated by control cultures. Heparan sulfate was the major glycosaminoglycan present in extracellular matrix of both control and methyl β-D-xylopyranoside-treated cultures; however, heparan sulfate proteoglycans of the extracellular matrix from methyl β-D-xylopyranoside-treated cultures were significantly smaller than those from control cultures. Methyl β-D-xylopyranoside treatment selectively inhibited incorporation of dermatan sulfate into extracellular matrix. The diminished size of the proteoglycans and the decreased amount of labeled glycosaminoglycans in matrix from methyl β-D-xylopyranoside-treated cultures suggests that these proteoglycans contain fewer glycosaminoglycan chains or fewer residues per chain. Alkaline borohydride digests of extracellular matrix proteoglycans from methyl β-D-xylopyranoside-treated cultures affords a very different profile from that obtained after digestion of extracellular matrix proteoglycans from normal cultures.

Kanwar *et al.*[13] have demonstrated that, in the presence of methyl β-D-xylopyranoside, synthesis of rat glomerular proteoglycan is significantly decreased as compared to that in normal tissue. Nevo *et al.*[14] demonstrated that, in the presence of methyl β-D-xylopyranoside, synthesis of chondroitin sulfate and keratan sulfate in embryonal chick cartilage decreased by ~60% as compared to non-

treated cartilage. Bovine corneal endothelial cells exposed to *p*-nitrophenyl β-D-xylopyranoside exhibited an 86% decrease in incorporation of labeled glycosaminoglycan into extracellular matrix. The synthesis of extracellular matrix dermatan sulfate or chondroitin sulfate containing proteoglycans was inhibited[15]. In addition, heparan sulfate proteoglycans present in extracellular matrix from methyl β-D-xylopyranoside-treated cultures were smaller and exhibited decreased charge density than comparable components from extracellular matrix from control cells[15].

In contrast to the effect of methyl β-D-xylopyranoside on incorporation of labeled proteoglycan into extracellular matrix, secretion of labeled glycosaminoglycans into the medium was stimulated ~2.5-fold in methyl β-D-xylopyranoside-treated cultures as compared to control cultures. Pronase experiments suggest that most of the glycosaminoglycans in the medium from methyl β-D-xylopyranoside-treated cells were free glycosaminoglycan chains, or glycosaminoglycan chains attached to very small peptides. In the presence of methyl β-D-xylopyranoside, secretion of dermatan sulfate or incorporation of dermatan sulfate into extracellular matrix was markedly inhibited. In addition, proteoglycan from extracellular matrix from methyl β-D-xylopyranoside-treated cultures were smaller than those isolated from extracellular matrix from control cultures.

These studies are in agreement with those of Kanwar *et al.*[13], Nevo *et al.*[14], and Robinson and Gospodarowicz[15] who have demonstrated methyl β-D-xylopyranoside-induced selective stimulation of free glycosaminoglycan chains into the medium in studies involving rat glomerular tissue, embryonal chick cartilage, and bovine corneal endothelial cells. As with human endothelial cells, secretion of dermatan sulfate was inhibited in methyl β-D-xylopyranoside-exposed bovine corneal endothelial cells[15].

The observation that, in the presence of methyl β-D-xylopyranoside, proteoglycan-associated glycosaminoglycan is markedly decreased in the extracellular matrix, and that stimulus of free glycosaminoglycan chains is exclusively into the medium, suggests that attachment of glycosaminoglycan chains to a core protein is a prerequisite for incorporation into extracellular matrix[15,23]. Studies demonstrate that β-D-xylopyranosides stimulate synthesis of free chondroitin chains[14,15,24–26] in a variety of cell types; however, the ability of β-D-xylopyranosides to stimulate free heparan sulfate chains is variable, depending on cell type[25,27,28]. Although most of the free glycosaminoglycan chains synthesized by methyl β-D-xylopyranoside-treated cultures were chondroitin sulfate, free heparan sulfate chains were also synthesized. In fact, the percentage of total radioactivity present in medium attributable to labeled heparan sulfate was slightly higher in methyl β-D-xylopyranoside-treated as compared to control cultures. Extracellular matrix from methyl β-D-xylopyranoside-treated cultures contained heparan sulfate almost exclusively. In the presence of methyl β-D-xylopyranoside, the percentage of total labeled proteoglycan present as heparan sulfate is considerably higher than in control cultures. These results suggest that methyl β-D-xylopyranoside can initiate

synthesis of heparan sulfate glycosaminoglycan chains and chondroitin sulfate glycosaminoglycan chains in cultures of human endothelial cells, but that not all of the free, labeled heparan sulfate is "trapped" by methyl β-D-xylopyranoside because some chains become bound to protein and are incorporated into extracellular matrix. These heparan sulfate proteoglycans are significantly smaller, however, than those isolated from matrix from control cultures.

Several studies have suggested that, in the presence of β-D-xylopyranosides, synthesis of other extracellular matrix components is also decreased. Loehmander *et al.*[29] and Schwartz and Dorfman[30] have demonstrated that, in the presence of methyl β-D-xylopyranoside, in addition to proteoglycan synthesis, collagen synthesis in cultured chondrocytes and collagen and also general protein synthesis in cultured fibroblasts is decreased. In human endothelial-cell cultures exposed to methyl β-D-xylopyranoside, incorporation of [^{3}H]proline into extracellular matrix is inhibited by about a third as compared to incorporation in the absence of methyl β-D-xylopyranoside. Proteoglycans are associated with collagen and fibronectin in the extracellular matrix and may have a central role in matrix formation[31–33]. Further experimentation is necessary to determine the effect of methyl β-D-xylopyranoside on collagen synthesis in human endothelial cells.

Proteoglycans are important structural components of the extracellular matrix that have been implicated in cell–substratum adhesion and detachment. Methyl β-D-xylopyranoside was used to investigate the effect of normal and proteoglycan-depleted matrix on cell adhesion and detachment. Cell-substratum detachment occurred at a significantly higher rate from proteoglycan-depleted matrix than from normal extracellular matrix. Conversely, cell adhesion to a proteoglycan-depleted matrix was perceptibly slower than to matrix containing significant amounts of proteoglycan. Several studies have implicated a role for heparan sulfate in cell-adhesion processes. Alpin *et al.*[34] have demonstrated that human amnion epithelial cells attach more readily to extracellular matrix than to plastic, and that cell adhesion may be inhibited by pretreatment of cells with heparin. Lark and Culp[7] have demonstrated that heparan sulfate is important in fibronectin-dependent 3T3 cell adhesion. Studies by Woods *et al.*[35] suggest that heparan sulfate proteoglycan may be involved in transmembrane cytoskeletal-matrix interactions.

Our studies suggest that cell adhesiveness is influenced by methyl β-D-xylopyranoside-induced alterations in extracellular matrix components. Further studies will be necessary to elucidate the components that mediate cell adhesion and cell detachment.

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